Lethality of mixtures of the anti-sea lice formulations, Salmosan® and Interox® Paramove®50 to mysid shrimp

Q. McCurdy, L.E. Burridge, M.C. Lyons

Science Branch, Maritimes Region
Fisheries and Oceans Canada
St. Andrews Biological Station
531 Brandy Cove Road, St. Andrews, NB
E5B 2L9

2013

Canadian Technical Report of Fisheries and Aquatic Sciences 3049

Fisheries and Oceans Canada | Pêches et Océans Canada

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts*.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques*.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Canadian Technical Report of
Fisheries and Aquatic Sciences 3049

September 2013

Lethality of mixtures of the anti-sea lice formulations, Salmosan® and Interox® Paramove®50 to mysid shrimp

by

[1]Q. McCurdy, L.E. Burridge, M.C. Lyons

Fisheries and Oceans Canada, Maritimes Region, St Andrews Biological Station,
531 Brandy Cove Road, St. Andrews, New Brunswick, Canada E5B 2L9

[1] Department of Geography and Environment, Mount Allison University, 62 York Street,
Sackville NB Canada E4L 1E2

This is the three hundred and seventh Technical Report of
the Biological Station, St. Andrews, NB

© Her Majesty the Queen in Right of Canada, 2013

Cat. No. Fs 97-6/3049E-PDF ISSN 1488-5379 (online version)

Correct citation for this publication:

McCurdy, Q., Burridge, L.E. and Lyons, M.C. 2013. Lethality of mixtures of the anti-sea lice formulations, Salmosan® and Interox® Paramove®50 to mysid shrimp. Can. Tech. Rep. Fish. Aquat. Sci. 3049: v + 11p.

TABLE OF CONTENTS

Abstract	iv
Introduction	1
Materials and Methods	3
Results	5
Discussion	7
Acknowledgements	8
References	9

ABSTRACT

McCurdy, Q., Burridge, L.E. and Lyons, M.C. 2013. Lethality of mixtures of the anti-sea lice formulations, Salmosan® and Interox® Paramove®50 to mysid shrimp. Can. Tech. Rep. Fish. Aquat. Sci. 3049: v + 11p.

Salmosan® and Interox®Paramove®50 are pesticides registered, or previously registered in Canada to combat infestations of parasitic copepods (sea lice) at Atlantic salmon (*Salmo salar*) aquaculture sites. Each contains a different active ingredient: azamethiphos in Salmosan® and hydrogen peroxide in Interox®Paramove®50. These formulations were used extensively at salmon aquaculture sites in southwest New Brunswick (SWNB) in 2010-2012 and it is plausible that in situ mixing of the two formulations could occur. While lethal thresholds have been determined for the individual pesticides on mysid shrimp species (*Praunus flexuosus* and *Mysis stenolepis*), to date, no study has determined the lethal thresholds for mixtures of the two formulations on these species. In this study mysid shrimp were exposed to either a mixture of Salmosan® and Interox® Paramove®50 or to Salmosan® followed by Interox® Paramove®50. Sequential exposure to recommended treatment concentrations resulted in mortality only in hydrogen peroxide exposed shrimp and exposure to mixtures resulted in LC_{50}'s the same as if the shrimp were exposed to Interox® Paramove®50 only (1200-1500 mg L^{-1}). Chemical analyses showed that when hydrogen peroxide was present in azamethiphos- spiked water the concentration of azamethiphos dropped more quickly than if no hydrogen peroxide was present.

RÉSUMÉ

McCurdy, Q., Burridge, L.E. and Lyons, M.C. 2013. Lethality of mixtures of the anti-sea lice formulations, Salmosan® and Interox® Paramove®50 to mysid shrimp. Can. Tech. Rep. Fish. Aquat. Sci. 3049: v + 11p.

Salmosan® et Interox® Paramove® 50 sont des pesticides homologués ou antérieurement homologués au Canada pour combattre les infestations de copépodes parasitaires (poux du poisson) dans les sites aquacoles de saumons de l'Atlantique (*Salmo salar*). La matière active du Salmosan® est l'azaméthiphos et celle du Interox® Paramove® 50 est le peroxyde d'hydrogène. Ces deux produits ont été largement utilisés dans des sites de salmoniculture dans le sud-ouest du Nouveau-Brunswick entre 2010 et 2012 et il est plausible que ces deux produits se soient mélangés *in situ*. Bien que les seuils létaux de ces pesticides aient été déterminés pour les mysis *Praunus flexuosus* et *Mysis stenolepis*, il n'existe jusqu'à présent aucune étude pour déterminer les seuils létaux d'un mélange des deux produits sur ces espèces. Dans le cadre de la présente étude, des mysis ont été exposées à un mélange de Salmosan® et de Interox® Paramove® 50 ou à un traitement de Salmosan® suivi de Interox® Paramove® 50. L'exposition séquentielle aux concentrations de traitement recommandées a seulement provoqué le décès de mysis exposées au peroxyde d'hydrogène, tandis que l'exposition aux produits mélangés a provoqué une CL_{50} identique à une exposition à l'Interox® Paramove® 50 tout seul (1 200 à 1 500 mg L^{-1}). Les analyses chimiques ont montré que dans de l'eau dopée avec l'azaméthiphos dans laquelle se trouve du peroxyde d'hydrogène, la concentration d'azaméthiphos chute plus rapidement qu'en l'absence de peroxyde d'hydrogène.

INTRODUCTION

Farmed salmon are stocked at densities of 14-17 kg per cubic meter in sea cages (ACFFA, 2010). Cultured salmon in the crowded conditions of aquaculture are susceptible to epidemics of infectious bacterial, viral and parasitic diseases (Haya et al., 2005). Sea lice are a group of ecto-parasites that are a problem for fish farms around the world (Burridge et al., 2010). Severe infestations of sea lice often result in costs to fish farmers either in loss of product or in the cost of combating the infestations (Haya et al., 2005). A number of pesticides have been used to combat sea lice infestations in Canada since sea lice first became a problem in 1994 when two species, *Lepeophtheirus salmonis* and *Caligus elongatus* infested salmon in southwest New Brunswick (*cf* Burka et al., 1997, Burridge, 2003, Burridge et al., 2010). Aquaculture pesticides can be applied by one of three methods: well boats, cage tarping, and cage skirting. All involve allowing affected fish to swim in a bath of pesticide-treated water, which, once the treatment is complete, is released to the surrounding environment (Burridge et al., 2003). Release of the effluent water, including the pesticide formulation to the surrounding water has raised concerns that unintended negative effects on non-target organisms might occur (Haya et al., 2005). Of particular concern in southwest New Brunswick (SWNB) is the potential for these pesticides to negatively impact other crustaceans such as the American lobster (*Homarus americanus*). Several studies have been conducted to determine the effects of these pesticides on lobster and on other non-target crustaceans (Burridge et al., 1999, Burridge et al., 2000; Ernst et al., 2001; Haya et al., 2001; Haya et al., 2005; Fairchild et al., 2010; Burridge, 2013; Burridge et al., Fisheries and Oceans, unpublished data).

Throughout 2009-2012 two anti-louse formulations were used to combat sea lice infestations in SWNB. Salmosan® 50WP formulation, Pest Control Products Act (PCPA) registration number 29466 (Health Canada, 2013a), contains 47.5% by weight azamethiphos (active ingredient (a.i.)). Azamethiphos is a neurotoxin, which targets the central nervous system, inhibiting the enzyme acetylcholinesterase (AChE). Acetylcholine propogates nerve signals across neural synapses and AChE breaks down acetylcholine thus stopping transmission of signals. When azamethiphos inhibits AChE, the neurons remain in an excited state, eventually causing irreparable nerve damage and death (Dutertre & Lewis, 2006).

Salmosan® is a water-soluble powder. In bath treatments, Salmosan® is used at a concentration of 100 $\mu g\ L^{-1}$ (as azamethiphos) in well boats and tarping, and 150 $\mu g\ L^{-1}$ when cages are surrounded with a skirt (Health Canada, 2013a). Salmosan® was fully registered as an anti-louse treatment in the 1990s (Burridge, 2003) and has had emergency registration with Health Canada until December 2012 (Health Canada, 2013a). Azamethiphos has a half-life of 8.9 days in water, and an octanol-water partition coefficient (log K_{ow}) of 1.05 which is relatively low (SEPA, 2005). The octanol-water partition coefficient is a benchmark value that is used to predict a chemical's persistence in the environment. A compound with a log K_{ow} less than 3 is not likely to persist in the environment. If log K_{ow} is greater than 5, an accumulation of the substance is likely (Beek et al., 2000).

The second formulation is Interox® Paramove®50, PCPA registration number 29783 (Health Canada 2013b), an emulsifiable concentrate containing 50% (by weight) hydrogen peroxide. Hydrogen peroxide acts by causing paralysis, peroxidation in organelle membranes, and inhibition of enzymes that replicate DNA (Cotran et al., 1989). Interox® Paramove®50 has emergency registration with Health Canada until June 2014 (Health Canada 2013b).

Interox®Paramove®50 is used in bath treatments at concentrations of 1.2 -1.8 g L^{-1} (as hydrogen peroxide) for up to 30 minutes (Health Canada, 2013b). Its effectiveness is highly dependent on water temperature, so concentrations are sometimes increased depending on the time of year and temperature (Treasurer et al., 1997). The half-life of stabilized hydrogen peroxide in seawater is about 7 days at 10°C with aeration (Bruno and Raynard, 1994) but degradation experiments in seawater with Interox® Paramove®50 currently underway at the St. Andrews Biological Station indicate that the half-life of hydrogen peroxide in that formulation is much longer (David Wong, Fisheries and Oceans Canada, pers. comm.). Similar to Salmosan®, Interox® Paramove®50 has a high affinity for water. The octanol-water partition coefficient for hydrogen peroxide is <1, indicating that it will not persist in the environment (HERA project, 2005).

The vast majority of anti-louse treatments in SWNB have been conducted with well boats or with use of full tarps (Dr. Michael Beattie, Province of New Brunswick pers. comm.). There is a chance that the respective owners of each site may want to take different approaches in mitigating the sea lice, choosing either Salmosan® or Interox® Paramove®50. In addition, in 2010 and 2011 some well boat treatments were conducted in which fish were treated with Salmosan®, the wells were flushed and then an Interox® Paramove®50 treatment applied (Dr. Michael Beattie, Province of New Brunswick, pers. comm.). Given the close proximity of cage sites in SWNB and the half-life of the active ingredients in these formulations, the possibility exists that non-target organisms could be exposed to both pesticides sequentially or at the same time.

The bays and inlets in SWNB are home to small crustaceans which may be as sensitive to anti-louse pesticides as the sea lice are. For example, mysid shrimp species (*Praunus flexuosus* and *Mysis stenolepsis)* that are indigenous to SWNB, are easily collected and held making them ideal for ecotoxicological studies. *P. flexuosus* is a non-native species originally from Scandinavia and like the native species, *M. stenolepis*, these mysids are now ubiquitous in the shallow coastal waters in SWNB. They are omnivorous but can be scavengers or cannibalistic and they provide a food source for higher trophic levels (Mauchline, 1980).

Previous studies at the St. Andrews Biological Station have shown that exposure of *Mysis stenolepis* to the recommended treatment concentration (100 $\mu g \cdot L^{-1}$) of azamethiphos (in the Salmosan® formulation) for 1h does not result in >50% mortality even when the shrimp were observed for a further 95 h (Burridge, 2013). Furthermore, the LC_{50} for hydrogen peroxide, based on measured concentrations (in the Interox® Paramove®50 formulation) was determined to be 973 mg L^{-1} with 95% confidence intervals (CI) of 668-1427 for a 1h exposure and a 95 h recovery period (Burridge, 2013). As stated earlier it is possible for

mixtures of Salmosan® and Interox® Paramove®50 to be present near cage sites soon after treatment. To date, there is no information available on the potential effects of these mixtures on non-target crustaceans.

In this study we examined the effects of sequential exposure of non-target crustaceans to Salmosan® followed by Interox®Paramove®50 as well as the effects of exposure to mixtures of the two formulations. Our objective was to determine whether two active ingredients have additive, synergistic or antagonistic effects and if so what this may mean in terms of risk assessment.

MATERIALS AND METHODS

Experimental animals

Mysid shrimp were collected using a beach seine net at Oven Head, NB which is several kilometers away from any active aquaculture sites. The mysids were transported to the St. Andrews Biological Station in 20 L buckets of sea water and held in a 60 L tank with flowing sand filtered sea water at ~14°C. Fresh mussels collected from Oven Head were shucked and fed to the mysids every two days.

Salmosan® was provided by Dr. Michael Beattie, Department of Agriculture, Aquaculture and Fisheries, Province of New Brunswick. Interox®Paramove®50 was provided by Mr. Ian Armstrong, Aqua Pharma Inc.

Experimental design

Two approaches were taken to examine the potential interactions between Interox®Paramove®50 and Salmosan®. In the sequential treatment experiment, the shrimp were exposed individually to either Salmosan® or Interox® Paramove®50 or sequentially to Salmosan® at the recommended treatment concentration (100 µg L^{-1} as azamethiphos) and then with Interox®Paramove®50 (1200 mg L^{-1} as hydrogen peroxide). This exposure regime mimics well boat treatments which took place in 2011 (Dr. Michael Beattie, Province of New Brunswick, personal communication).

For the individual and mixture treatment experiment, shrimp were exposed individually to either Salmosan® or Interox® Paramove®50 or to a mixture of Salmosan® and Interox® Paramove®50 to mimic a situation where, after operational treatments, the two products might be present in the near-cage or near-well boat environment at the same time. All animals exposed to the anti-louse formulations for 1 hr were monitored for a further 95 hr to assess delayed mortality.

Sequential treatments

Fifteen mysid shrimp were held individually in 10 mL glass beakers filled with ~ 7.5 mL of untreated water (controls) or water with Salmosan® at a concentration of 100 µg L^{-1} as azamethiphos. After 1 hr the mysids were transferred to clean flowing seawater for 20 minutes and then moved to ~7.5 mL of untreated water (controls) or water spiked with Interox®, Paramove®50 at a concentration of 1200 mg L^{-1} as hydrogen peroxide. After all exposures the mysids were transferred to mesh containers held in flowing seawater and

monitored for 95 hours post treatment. The shrimp were assessed at 1, 3, 6, 12, 24, 48, 72 and 95 hr for mortality. In addition, some behavioural responses (swimming activity, position in the water column and orientation) were assessed and recorded. Water temperature ranged from 12.6°C to 13.6°C.

Individual and mixture treatments

The bioassays in which the shrimp were exposed to only one formulation or to mixtures of the two formulations were conducted 10 months after the bioassays in which the shrimp were exposed to the formulations sequentially. In these assays ten mysid shrimp were transferred from the holding tank to 500 ml glass beakers filled to 400 ml with various concentrations of azamethiphos, hydrogen peroxide or mixtures. The exposure concentrations are shown in Table 1. The shrimp were exposed for 1 h then were transferred to mesh containers and held in a flow-through seawater bath and monitored for a further 95 h. Mysids from all exposures were checked at 1, 3, 6, 12, 24, 48, 72, and 96 h. Information was collected on mortalities, escapees, cannibalism, dissolved oxygen and temperature. Each bioassay was conducted three times. Water temperature ranged from 14.1°C to 15.2°C.

Table 1. Target concentrations of azamethiphos and/or hydrogen peroxide in lethality studies with mysid shrimp.

Salmosan®	Interox® Paramove®50	Mixtures of Salmosan® and Interox® Paramove®50	
Azamethiphos exposure concentrations ($\mu g\ L^{-1}$)	Hydrogen peroxide exposure concentrations ($mg\ L^{-1}$)	Azamethiphos exposure concentrations ($\mu g\ L^{-1}$)	Hydrogen peroxide exposure concentrations ($mg\ L^{-1}$)
0	0	0	0
13	195	13	195
22	325	22	325
36	540	36	540
60	900	60	900
100	1500	100	1500
	2500	100	2500

Water analysis

Azamethiphos

Water samples (40 ml) were taken at T= 0 and T=1 h and preserved with 5 ml dichloromethane (DCM). The water samples were placed on a tumble mixer for 1 h to ensure DCM was thoroughly mixed, then moved to a refrigerator until analyzed. On removal from the refrigerator an additional 5 mL of DCM was added to each sample (DCM total now 10 mL) and the samples were mixed for one hour on a rotary drive mixer. The samples were allowed to sit for at least one hour and 9 mL of DCM was collected from each sample. The extracts were taken to dryness under nitrogen at 40°C on a TECHNE Sample Concentrator and DB-3D Dri Block. One mL of acetonitrile was added and mixed using a vortex mixer. Each sample was transferred to a 2 mL sample vial for High Performance Liquid Chromatography (HPLC) analysis. Blank water samples were extracted in the same manner and extraction was confirmed using freshly spiked seawater samples.

All samples, calibration standards, as well as quality control samples were analysed using HPLC equipped with an Ultraviolet/Visable (UV/Vis) detector under the following analytical conditions:

Mobile Phase: Water; acetonitrile (68:32) at 1.2 mL per minute
Column: Supelco LC-19-DB (250 x 4.6 mm id)
Column Temperature: 40°C
Injection Volume: 20µL
UV Wavelengths: Analytical – 295nm with 4nm bandwidth
Reference – 360nm with 100nm bandwidth

Hydrogen peroxide

Water samples were analysed for presence and concentration of hydrogen peroxide using titration with a cerium sulphate/sulfuric acid mixture as prescribed by Aqua Pharma Inc. (Ian Armstrong, personal communication). Briefly, water samples were added dropwise to the cerium sulphate/sulfuric acid mixture until all colour disappeared (yellow to clear). The volume of water added is proportional to the quantity of hydrogen peroxide present.

LC_{50} determination

Measured concentrations of azamethiphos or hydrogen peroxide were used to estimate LC_{50}'s. Mortality observations at 24 h and 96 h were used to calculate the estimates. The 1 h exposure LC_{50} estimates (24 and 96 h) with 95% CI were determined according to Stephan (1977) using the Toxstats program. All LC_{50}'s were calculated using a Spearman-Karber analysis with the exception of one 24 h LC_{50} estimate for Interox®Paramove®50 (hydrogen peroxide) which was estimated using a probit analysis. In sequential- exposure bioassays, replication was by individual container. In the mixture experiments the LC_{50} estimates were averaged and a confidence interval calculated for the average.

RESULTS

Sequential treatments

There were no mysid mortalities in the control or 1 h Salmosan® only treatments over the 96 hours. Thirteen of the fifteen mysids died in the 1 h Interox® Paramove®50-only treatment over the 96 hours and fourteen of the fifteen mysids died in the sequential treatment of 1 h Salmosan® and 1 h Interox® Paramove®50. Mortalities were first seen as early as three hours after the beginning of the exposures. The measured water concentration for hydrogen peroxide was ~1400 mg L^{-1} which was slightly higher than the 1200 mg L^{-1} nominal concentration.

Individual and mixture treatments

Measured concentrations of hydrogen peroxide in water samples are presented in Table 2. The measured concentrations of hydrogen peroxide were higher than the nominal concentrations which was consistent with the measured versus nominal concentration in the sequential exposure. The difference between the measured concentrations of

hydrogen peroxide in the Interox® Paramove®50 test and in the Salmosan®+Interox® Paramove®50 test was negligible as shown in the percent difference column of Table 2.

Table 2. Measured concentrations of hydrogen peroxide in exposure water collected from bioassays compared to the predicted (nominal) concentration.

Time (hours)	Nominal concentration (mg L^{-1})	Measured hydrogen peroxide in Interox® Paramove®50 only (mg L^{-1})	Measured hydrogen peroxide in Salmosan®+Interox® Paramove®50 mixture (mg L^{-1})	Percent difference between the hydrogen peroxide conc. in the Paramove® only treatment and the mixed treatment (%)
0	540	628	619	1.43
0	1500	1712	1679	1.93
0	2500	2866	2802	2.23
1	540	622	602	3.22
1	1500	1679	1646	1.96
1	2500	2772	2656	4.18

Measured concentrations of azamethiphos in water samples are presented in Table 3. The average measured concentrations of azamethiphos are very close to the nominal concentrations for the treatments of Salmosan® only. This is not the case for the Salmosan®+Interox® Paramove®50 mixtures. The percent difference between the measured azamethiphos in the Salmosan® only exposure compared to that of the Salmosan®+Interox® Paramove®50 mixture after a 1-h exposure is 54% in the lower concentration, and 65% in the highest concentration.

LC_{50} estimates could not be calculated for all tests. The LC_{50} calculation method needs at least one concentration with greater than 50% mortality, and at least one concentration with less than 50% mortality. This was only obtained in 4 of the 9 tests. These four tests included two Interox® Paramove®50 tests and two Salmosan® + Interox® Paramove®50 tests. An LC_{50} estimate could not be calculated for the Salmosan® only trials. LC_{50}'s are shown in Table 4.

Table 3. Measured concentrations of azamethiphos in exposure water collected from bioassays compared to the predicted (nominal) concentration.

Time (hours)	Nominal concentration (µg L^{-1})	Measured azamethiphos in Salmosan® only (µg L^{-1})	Measured azamethiphos in Salmosan®+Interox® Paramove®50 (µg L^{-1})	Percent difference between the azamethiphos conc. in the Salmosan® only treatment and the mixed treatment (%)
0	36	31	31	0.00
0	100	100	89	11.00
1	36	35	16	54.29
1	100	97	34	64.95

Table 4. Lethality (24 h and 96 h LC50's) of hydrogen peroxide (in Interox® Paramove®50) to mysid shrimp with 95% CI. Mysids were exposed for 1 hour then monitored for a further 95 h.

Formulation	Time	Mean LC_{50} (mg L^{-1})	95% CI
Salmosan®	24 h	ND*	ND
	96 h	ND	ND
Interox® Paramove®50	24 h	1650	1201-2141
	96 h	1222	958-1558
Salmosan®+ Interox® Paramove®50	24 h	1730	1368-2190
	96 h	1506	1150-1974

* ND – Not determined as < 50% of exposed shrimp died

DISCUSSION

The results of the sequential treatment of mysid shrimp to Salmosan® at a concentration of 100 µg L^{-1} as azamethiphos followed by Interox® Paramove®50 at a concentration of 1200 mg L^{-1} as hydrogen peroxide showed that there were no additive, synergistic or antagonistic effects for the two formulations. Lethality results were very similar to the individual treatments of either Salmosan® or Interox® Paramove®50. Sequential exposure to recommended treatment concentrations resulted in mortality of mysid shrimp only after exposure to hydrogen peroxide.

Exposure to mixtures resulted in LC_{50}'s in the same range as if the shrimp were exposed to Interox® Paramove® 50 only (1222 and 1506 mg L^{-1} hydrogen peroxide) after 96 h. The 24 h LC_{50} for hydrogen peroxide in the Interox® Paramove® 50 individual treatment was compared to 24 h LC_{50} for hydrogen peroxide in the mixed treatment (Salmosan®+ Interox® Paramove® 50) and the difference between the two (1650 and 1730 mg L^{-1} hydrogen peroxide) was only 4.6%. Confidence intervals show considerable overlap (Table 4). The 96 h LC_{50}'s were lower than the 24 h LC_{50}'s indicating greater mortality over time. Burridge (2013) reported a 96 h LC_{50} of 973 mg L^{-1} hydrogen peroxide for a 1 h exposure to mysid shrimp. Although his 96 h LC_{50} estimate was lower, the 95% CI's (668-1427) show overlap with the values reported in table 4.

The measured concentrations of hydrogen peroxide in the Interox® Paramove®50 exposure water were higher than the nominal concentrations. This is consistent with results in the sequential treatment where measured concentrations of hydrogen peroxide were consistently higher than nominal concentrations. Only the tests with a nominal concentration of 2500 mg L^{-1} (~2660-2850 mg L^{-1} hydrogen peroxide measured) yielded results that killed 100% of the mysids. This is consistent with previous 1 h exposures of mysid shrimp where 1500 mg L^{-1} hydrogen peroxide did not kill 100% of the mysids but 3000 mg L^{-1} hydrogen peroxide did (Burridge, 2013).

Results from the mixture treatments (Salmosan®+Interox® Paramove®50) showed that when Interox® Paramove®50 was present in water with Salmosan®, the measured azamethiphos in the Salmosan® was reduced. Analysis of the treatment water showed a steady concentration of hydrogen peroxide throughout the 1 h treatment while azamethiphos concentration was decreased by roughly 60% (Table 3). HPLC analysis of treatment water from the Salmosan® only exposures showed that azamethiphos concentration was only reduced by an average of 3% after 1 h (Table 3).

Hydrogen peroxide is a strong oxidizer and has been investigated in the oxidation of organophosphates by using Fenton's reagent (Dowling and Lemley, 1995; Doong and Chang, 1998). We hypothesize that azamethiphos in the mixed treatment water was degraded by an oxidation reaction with hydrogen peroxide. We speculate that the relatively fast reduction of azamethiphos concentration in the presence of hydrogen peroxide could decrease its effectiveness not only on sea lice but other sensitive non-target organisms.

The risk of hydrogen peroxide affecting non-target organisms when it is mixed with azamethiphos is similar to hydrogen peroxide on its own, for both the 24 h and 96 h LC_{50} estimates. In a real world scenario, the effect of mixing the two compounds effectively lowers the risk of azamethiphos to non-target organisms, but the effect of hydrogen peroxide remains constant.

In conclusion, hydrogen peroxide lowered the concentration of azamethiphos over a 1 h treatment and it effectively lowers the risk for non-target species that may have lethal responses to azamethiphos. Various life stages of lobster die in 48 h exposures to azamethiphos (Burridge et al., 1999). If the presence of hydrogen peroxide reduces the concentration of azamethiphos over a 1 hour period, that may result in fewer lobster mortalities if mixtures of the two were used in treatments. Burridge (2013) reported a 1 h LC_{50} of azamethiphos for adult lobster as 24.8 $\mu g\ L^{-1}$. It is unlikely that a plume from a Salmosan® treatment of 100 $\mu g\ L^{-1}$ (as azamethiphos) oxidized by hydrogen peroxide by more than 50% and then further diluted in the environment would reach adult lobster at lethal concentrations.

These data suggest that mixing these formulations as a single treatment option either in a tarped cage or in a well boat would not increase efficacy. The presence of hydrogen peroxide in an effluent from one treatment, if mixed with azamethiphos in an active treatment, could potentially decrease the effectiveness of the Salmosan® treatment. Sequential exposure or exposure to mixtures had no additive or synergistic effect on the non-target mysid shrimp.

ACKNOWLEDGEMENTS

The authors wish to thank Ken MacKeigan for analytical support.

REFERENCES

ACFFA, 2010. At: http://atlanticfishfarmers.com/fish-stocking.html Accessed May 2013.

Beek, B., Bohling, S., Bruckmann, U., Franke, C., Johncke, U. and Studinger, G., 2000. The assessment of bioaccumulation, p. 239-276. In B. Beek [ed.]. The handbook of environmental chemistry Vol. 2 (Part J): Bioaccumulation new aspects and developments.

Bruno, D.W. and Raynard, R.S., 1994. Studies on the use of hydrogen peroxide as a method for the control of sea lice on Atlantic salmon. Aquaculture International 2.10-8.

Burka, J.F., Hammell, K.L., Horsburg, T.E., Johnson, G.R., Rainie, D.J. and Speares, D.J., 1997. Drugs in salmonid aquaculture – A review. J. Vet. Pharmacol. Ther. 20: 333-349.

Burridge, L.E., 2003. Chemical use in marine finfish aquaculture in Canada: A review of current practices and possible environmental effects. Canadian Technical Report of Fisheries and Aquatic Sciences 2450 [ix + 131pp].

Burridge, L., 2013. A review of potential environmental risks associated with the use of pesticides to treat Atlantic salmon against infestations of sea lice in southwest New Brunswick, Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2013/050. iv + 25 p.

Burridge, L.E., Haya, K., Zitko, V. and Waddy, S., 1999. The lethality of Salmosan® (azamethiphos) to American lobster (*Homarus americanus*) larvae, post-larvae, and adults. Ecotoxicol. Environ. Saf. 43: 165-169.

Burridge, L.E., Haya, K., Waddy, S.L., Wade, J., 2000. The lethality of anti-sea lice formulations Salmosan® (azamethiphos) and Excis® (cypermethrin) to stage IV and adult lobsters (*Homarus americanus*) during repeated short-term exposures. Aquaculture 182: 27-35.

Burridge, L., Weis, J., Cabello, F., Pizarro, J. and Bostick, K., 2010. Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture 306: 7-23.

Cotran, R.S., Kumar, V., and Robbins, S.L., 1989. Pathological basis of disease; 4 ed.; Saunders: Toronto.

Doong, R., Chang, W., 1998. Photoassisted iron compound catalytic degradation of organophosphorous pesticides with hydrogen peroxide. Chemosphere 37(13): 2563-2572.

Dowling, K.C., Lemley A.T., 1995. Organophosphate insecticide degradation by non-amended and cupric ion-amended Fenton's reagent in aqueous solution. J. Environ. Sci. Health, Pt B: Pestic , Food Contam , Agric Wastes B30(5): 585-604.

Dutertre, S., & Lewis, R., 2006. Toxin insights into nicotinic acetylcholine receptors. Biochem. Pharmacol., 72(6): 661-670.

Ernst, W., Jackman, P., Doe, K., Page, F., Julien, G., Mackay, K., and Sutherland, T., 2001. Dispersion and toxicity to non-target aquatic organisms of pesticides used to treat sea lice on salmon in net pen enclosures. Mar. Poll. Bull. 42: 433-444.

Fairchild, W.L., Doe, K.G., Jackman, P.M., Arsenault, J.T., Aubé, J.G., Losier, M., Cook, A.M., 2010. Acute and chronic toxicity of two formulations of the pyrethroid pesticide deltamethrin to an amphipod, sand shrimp and lobster larvae. Can. Tech. Rep. Fish. Aquat. Sci. 2876: vi + 34.

Haya, K., Burridge, L.E., Chang, B.D., 2001. Environmental impact of chemical wastes produced by the salmon aquaculture industry. ICES J. Mar Sci. 58:492-496.

Haya, K., Burridge, L.E., Davies, I.M., Ervik, A., 2005. A review and assessment of environmental risk of chemicals used for the treatment of sea lice infestations of cultured salmon. In: Hargrave B. (Ed) Handbook of Environmental Chemistry Volume 5: Water Pollution, Part M 305-341.

Health Canada, 2013a. Registration status of Salmosan at: http://pr-rp.hc-sc.gc.ca/pi-ip/index-eng.php Accessed June 11, 2013.

Health Canada, 2013b. Registration status of Interox® Paramove 50® at: http://pr-rp.hc-sc.gc.ca/pi-ip/index-eng.php Accessed June 12, 2013.

HERA project. 2005. Human & Environmental Risk Assessment on ingredients of household cleaning products. http://www.heraproject.com/files/36-F-05-Shor_H2O2_version1.pdf Accessed February 20, 2012.

Mauchline, J. (1980). The biology of mysids and euphausiids. In: Blaxter, J H. S., Russell, F. S., Yonge, C. M. (eds.) Advances in marine biology, Vol. 18. Academic Press, New York, p 1-369

Scottish Environmental Protection Agency. (SEPA), 2005. See attachments in: Fish farm manual.
http://www.sepa.org.uk/water/water_regulation/regimes/aquaculture/marine_aquaculture/fish_farm_manual.aspx Accessed Oct 17, 2011.

Stephan, C.E., 1977. Methods for Calculating an LC_{50}. Aquatic toxicology and hazard evaluation, ASTM STP 634, F. L. Mayer and J. L. Hamelink, Eds. , American Society for Testing and Materials, 1977, pp. 65-84.

Treasurer, J.W., Grant, A., 1997. The efficacy of hydrogen peroxide for treatment of farmed Atlantic salmon, *Salmo salar* L. infested with sea lice (Copepoda: Caligidae). Aquaculture 148, 265-275.